Callus Culture in *Catharanthus roseus* (L.) G. Don: A Survey of Induction Strategies, Growth Optimization, and Biotechnological Applications

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Abstract. *Catharanthus roseus* (L.) G. Don owes its place among medicinally important plants to a chemical repertoire of more than 200 bioactive compounds, among them the terpenoid indole alkaloids vincristine and vinblastine, both still central to cancer chemotherapy regimens decades after their discovery. Because these molecules occur in only trace amounts in the intact plant, and because pharmaceutical demand for them has not slackened, workers in the field have spent several decades looking for production routes that do not depend entirely on field cultivation. Callus culture has proved to be one of the more workable answers: an in vitro system useful not only for propagation and germplasm conservation but, under favourable conditions, for raising the yield of secondary metabolites as well. Whether a given culture succeeds or not tends to come down to a small set of interacting variables — which explant is chosen, what the medium is made of, which combination of growth regulators is applied, and the physical conditions under which the tissue is incubated. Hormones in particular govern induction, proliferation and the eventual fate of the callus, while nutrient supply, pH, light regime and temperature shape both how much biomass accumulates and how actively metabolites are synthesised. This review pulls together what is currently known about callus culture in C. roseus, starting with the underlying biology of callus formation and the dedifferentiation–redifferentiation cycle, moving through the practical factors that govern initiation and growth, and then considering how researchers typically characterise the callus they obtain. It goes on to examine applications in alkaloid production, whole-plant regeneration and elicitor-based strategies for boosting metabolite output, before closing with the obstacles that still limit the field and the directions from which further progress seems most likely, particularly metabolic engineering and related biotechnological tools. Read together, the available literature points to callus culture as one of the more durable platforms for the sustainable production of pharmaceutically valuable compounds from C. roseus.

Keywords: *Catharanthus roseus*, Callus culture, Plant tissue culture, Terpenoid indole alkaloids, Vincristine, Vinblastine, Secondary metabolites, Plant growth regulators, Explants, Elicitation, Morphogenesis.

1 Introduction

Catharanthus roseus (L.) G. Don — better known outside the lab as the Madagascar periwinkle — belongs to the family Apocynaceae and owes its medicinal reputation to a set of terpenoid indole alkaloids (TIAs) that includes vincristine, vinblastine, ajmalicine, serpentine and catharanthine (Kabesh et al., 2015). Two of these, vincristine and vinblastine, remain front-line chemotherapeutic agents used against leukaemia, Hodgkin's lymphoma and breast cancer (Mishra & Verma, 2017), while the plant as a whole also shows antidiabetic, antimicrobial, antioxidant, antihypertensive and anti-inflammatory activity, a breadth of pharmacological effects that has kept it firmly on the radar of pharmaceutical researchers (Aslam et al., 2010). The trouble is that extracting these compounds from field-grown plants runs into several practical limits at once: alkaloid concentrations are naturally low, yields swing with season and growing conditions, cultivation takes time, and wild populations are increasingly overharvested. Metabolite content also differs from one plant part and developmental stage to the next, which further undermines the consistency of conventional extraction (Verpoorte et al., 2002). None of

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this is new — it is precisely why plant tissue culture, and callus culture specifically, has become such a well-established alternative for conserving genetic material, propagating plants, and pushing secondary metabolite yields higher (Loyola-Vargas & Ochoa-Alejo, 2018). In practice, callus culture means inducing an unorganised, actively dividing mass of cells from an explant under sterile, controlled conditions, after which growth and metabolism can be steered fairly precisely by adjusting the nutrient medium, the growth regulators supplied, and the physical environment (Efferth, 2019). Done well, this kind of system supports scalable, repeatable metabolite production alongside genetic transformation work and germplasm banking (Halder & Jha, 2020). For

C. roseus specifically, callus has been raised successfully from a fairly wide range of tissues — leaves, stems, roots, hypocotyls and floral material among them (Singh et al., 2011; Bansal et al., 2023; Desouky et al., 2026). Even so, how efficiently a given explant calluses, and how fast the resulting tissue grows, depends heavily on the source tissue, the genotype, the basal medium, and the auxin and cytokinin levels used, and different labs report considerable variation on all of these fronts (Singh et al., 2011; Desouky et al., 2026). A great many protocols have been published over the years, yet the field still lacks anything close to a standardised, reliably reproducible method, and that gap remains one of the more persistent obstacles to optimising callus systems in this species.

2 Botanical Overview of Catharanthus roseus

2.1 Taxonomy

Domain — Eukaryota

Kingdom — Viridiplantae

Phylum — Streptophyta

Class — Magnoliopsida

Order — Gentianales

Family — Apocynaceae

Genus — Catharanthus

Species — *Catharanthus roseus*

2.2 Distribution and Habitat

Catharanthus roseus (L.) G. Don goes by several regional names — Nityakalyani, Cayenne jasmine, old maid, sadabahar, batla hoo, nayantara and barmasi among them (Paarakh et al., 2019) — and traces its origin to Madagascar, the island nation off the East African coast (Mishra & Verma, 2017). Its ornamental appeal combined with its medicinal value has carried it well beyond that original range, and it is now grown throughout the tropics and subtropics (Paarakh et al., 2019). In India specifically, it turns up both as a garden ornamental and as a field crop grown expressly for alkaloid extraction (Nejat et al., 2015). The species does best in warm climates and copes with a fairly broad range of habitat conditions (Paarakh et al., 2019), preferring well-drained sandy loam soils of moderate fertility and full sun for vigorous flowering (Paarakh et al.,

2019). It also tolerates drought and heat reasonably well, which goes some way toward explaining why it has spread so successfully through tropical regions worldwide (Nejat et al., 2015). This tolerance for varied growing conditions is, in large part, what has made it such a widely cultivated ornamental and medicinal species (Nejat et al., 2015).

2.3 Morphological Characteristics

C. roseus is a perennial herb, usually reaching somewhere between 1 and 2.5 feet in height (Paarakh et al., 2019). Its leaves are simple, glossy, hairless and oval to oblong, arranged in an opposite decussate pattern (Das & Sharangi, 2017), typically 2.5–9.5 cm long by 1.0–3.5 cm wide, with a noticeably pale midrib (Kabesh et al., 2015). Petioles run 1.0–1.8 cm and range from green to reddish. Flowers are borne in a racemose inflorescence (Nejat et al., 2015), about 4–5 cm across, usually white or pink with a contrasting central eye that can appear purple, red, pale yellow or white (Paarakh et al., 2019). Seeds are small — 2 to 3 mm — black, and cylindrical to oblong (Sharma et al., 2024; Nejat et al., 2015).

2.4 Economic and Medicinal Importance

C. roseus earns its economic importance almost entirely from its medicinal chemistry (Mishra & Verma, 2017). Its tissues carry a broad mix of indole alkaloids, flavonoids, phenolic acids and terpenoids, and this chemical diversity underlies most of its pharmacological uses (Van der Heijden et al., 2004; Gupta et al., 2013; Pandey et al., 2011; Facchini et al., 2012). Traditional and modern practitioners alike have used it for menorrhagia, rheumatism, dyspepsia, indigestion, dysmenorrhoea, diabetes, hypertension and other menstrual complaints (Kabesh et al., 2015), and its alkaloid content also gives it demonstrated efficacy against certain cancers (Goswami et al., 2024). Beyond that, it has been applied to skin conditions and bleeding diarrhoea, and shows sedative and antiviral activity as well (Kabesh et al., 2015). Anti-inflammatory, antioxidant and immunomodulatory effects round out the picture, which is part of why the plant sits comfortably in both folk and clinical pharmacology (Goswami et al., 2024). Roughly 130 distinct alkaloids have been identified in the species (Das & Sharangi, 2017), several of which — catharanthine, leurosine sulfate, lochnerine, tetrahydroalstonine, vindoline and vindolinine among them — lower blood glucose, while vincristine and vinblastine remain the standout anticancer compounds (Nisar et al., 2016).

3 Principles of Callus Culture

3.1 Definition and Concept of Callus

The word “callus” comes from the Latin *callum*, meaning hard tissue — in medicine it refers to thickened skin, but in plant tissue culture it means something rather different: an unorganised mass of proliferating, undifferentiated cells that arises from an explant under in vitro conditions (Ikeuchi et al., 2013; Efferth, 2019). This tissue typically forms when differentiated cells regain meristematic activity (Wawrosch & Zotchev, 2021). Two broad classification schemes are commonly applied. On the basis of texture, callus is described as either compact — firm and densely packed — or friable, meaning soft and loosely arranged, breaking apart readily into smaller aggregates and therefore well suited to starting cell suspension cultures (Efferth, 2019). On the basis of developmental potential, callus is either embryogenic, capable of forming

somatic embryos and regenerating whole plants, or non-embryogenic, which simply proliferates without that capacity (Fehér, 2019).

3.2 Cellular Dedifferentiation and Redifferentiation

Differentiation, in the strict developmental sense, is the process through which unspecialised or meristematic cells acquire the structure and function needed for a specific role in the plant body (Sugiyama, 1999). Dedifferentiation runs the process in reverse: mature, specialised cells revert to a meristematic state and regain the ability to divide actively. In culture, this is usually triggered by the mechanical wounding that occurs when an explant is excised, together with an appropriate dose of plant growth regulators, and the outcome is the disorganised cell mass we call callus (Ikeuchi et al., 2013). Redifferentiation is the reverse step again — dedifferentiated cells reorganise into shoots, roots or somatic embryos when the culture conditions favour it (Sugiyama, 1999). Together, these two processes underpin essentially all successful callus induction and plant regeneration work, and they set the ceiling on how efficient an in vitro system can be for enhanced secondary metabolite production in C. roseus.

3.3 Factors Governing Callus Initiation and Proliferation

How readily and how vigorously callus initiates and proliferates depends on several interacting variables: the explant source, genotype, medium composition, growth regulator regime, and the surrounding environmental conditions. Because these factors interact rather than acting independently, the efficiency of induction and the subsequent growth or morphogenic behaviour of the tissue can vary considerably even within a single protocol (George et al., 2008).

4 Establishment of Callus Cultures in Catharanthus roseus

Setting up a callus culture in *C. roseus* comes down to three linked decisions: which explant to use, how to sterilise it, and how to formulate the medium. The explant choice matters a great deal here, since hormone levels, physiological state, MS medium strength, and the type and concentration of carbon source all interact with tissue origin to determine how efficiently callus forms and grows. Getting this first step right is, in effect, the foundation for any reliable induction protocol (Verma et al., 2012; Desouky et al., 2026).

4.1 Selection of Explants

Explant choice is arguably the single most consequential decision in setting up a *C. roseus* callus culture, since different tissues bring very different levels of morphogenic competence and responsiveness to in vitro treatment. Researchers have worked with leaves, stems, roots, shoot tips, hypocotyls, seeds and floral organs, and callogenic efficiency across these tissues tracks with endogenous hormone balance, physiological age, genotype and the general metabolic state of the donor plant (Verma et al., 2012; Rahman et al., 2019; Bansal et al., 2023; Taha et al., 2008; Desouky et al., 2026).

Explant type	Major findings	Potential applications	Representative studies
Leaf	One of the most widely used explants; shows good callogenic potential on MS medium with auxin–cytokinin combinations. Leaf-derived callus has also supported secondary metabolite enhancement studies.	Callus induction, alkaloid production, elicitation studies	Taha et al. (2008); Rahman et al. (2019)
Stem	Tends to outperform leaf tissue in callus induction frequency and biomass accumulation across several PGR treatments.	Large-scale callus production, biomass generation, alkaloid accumulation studies	Desouky et al. (2026)
Shoot tip	High callogenic and regenerative potential; associated with enhanced direct shoot regeneration and higher total alkaloid content.	Micropropagation, regeneration studies, alkaloid production	Taha et al. (2008)
Hypocotyl	Juvenile tissue with high morphogenic competence and regenerative ability, suited to transformation work.	Genetic transformation, regeneration systems	Wang et al. (2012)
Root	Capable of callus induction under suitable conditions; some studies report relatively high total alkaloid accumulation.	Secondary metabolite production, comparative callogenesis	Taha et al. (2008)
Anther	High induction frequencies; investigated as a source of novel bioactive compounds.	Haploid culture, phytochemical studies	Bansal et al. (2023)
Seeds	Used successfully for callus induction; resulting callus served as a source of bioactive compounds for nanoparticle synthesis.	Green synthesis of silver nanoparticles, biotechnological applications	Osibe et al. (2018)

Table 1. Explants used for callus induction in *Catharanthus roseus* and their reported responses

4.2 Surface Sterilization Protocols

Getting aseptic cultures established in *C. roseus* depends on thorough surface sterilisation before the explant ever reaches the medium. A handful of disinfectants show up repeatedly across the literature — 70% ethanol, sodium hypochlorite, mercuric chloride, calcium hypochlorite and various commercial bleach formulations — and explants are typically washed under running tap water with a mild detergent beforehand to strip away dust and surface debris (George et al., 2008; Verma et al., 2012; Kalidass et al., 2010). Exact concentrations and exposure times shift depending on how delicate the tissue is: a fairly common regime is 30–60 seconds in 70% ethanol followed by 2–5 minutes in 0.1% mercuric chloride, or alternatively 5–15 minutes in 1–2% sodium hypochlorite (Malabadi et al., 2012; Kaya & Aki, 2013). Push the exposure too far and the tissue itself is damaged; keep it too short and contamination becomes likely, so sterilisation protocols generally need fine-tuning for each explant type — leaves, stems, roots and floral tissue all behave somewhat differently in this respect (Taha et al., 2008; Wang et al., 2012).

4.3 Culture Media Used

Murashige and Skoog (MS) medium remains, by a wide margin, the basal medium of choice for inducing and maintaining callus in this species. Its balanced mix of macro- and micronutrients, vitamins and inorganic salts supports rapid division and steady callus growth (Murashige & Skoog, 1962), and most published protocols rely on full-strength MS supplemented with a suitable auxin–cytokinin pairing (Taha et al., 2008; Desouky et al., 2026; Bansal et al., 2023). pH is generally set to 5.7–5.8 before autoclaving to keep nutrients available and tissue growth on track. Sucrose at around 6% usually serves as the carbon source, with agar at 6–8 g L^{-1} providing the gelling matrix (George et al., 2008; Phillips & Garda, 2019; Verma et al., 2012).

4.4 Strength of Culture Media

Medium strength turns out to matter almost as much as its composition. Full-strength MS tends to give the largest callus biomass simply because more nutrients are on hand, but reduced-strength formulations can sometimes push alkaloid accumulation higher by shifting the nutrient balance and, with it, cellular metabolism (Verma et al., 2012). Mishra et al. (2018) found that raising nutrient strength from 0.5× to 1.0× MS improved both callus biomass and alkaloid output, but pushing beyond 1.0× gave only marginal further gains — a reminder that nutrient concentration is worth tuning to the specific goal of the culture rather than simply maximised.

5 Influence of Plant Growth Regulators on Callus Induction

Plant growth regulators (PGRs) sit at the centre of callus induction, proliferation and differentiation in *C. roseus*. The balance struck between auxins and cytokinins in the medium is really what drives whether and how callus forms in the first place (George et al., 2008). Among auxins, 2,4-dichlorophenoxyacetic acid (2,4-D) and α-naphthaleneacetic acid (NAA) dominate the literature, while 6-benzylaminopurine (BAP) and kinetin are the cytokinins used most often. Combining an auxin with a cytokinin generally outperforms using either alone, a pattern that shows up repeatedly across independent studies (Verma et al., 2012; Desouky et al., 2026; Taha et al., 2008; Bansal et al., 2023). Media built around 2,4-D with kinetin, or NAA with BAP, tend to give particularly strong callogenic responses across a range of explants (Taha et al., 2008; Rahman et al., 2019; Desouky et al., 2026), though the exact response still shifts with explant type, genotype and the broader culture conditions (George et al., 2009; Aslam et al., 2010). Mechanistically, auxins push cells toward dedifferentiation and division, while cytokinins drive proliferation and shape the resulting callus morphology — which is why getting the auxin-to-cytokinin ratio right is so central to achieving efficient induction and good biomass yield in this species (George et al., 2008; Phillips & Garda, 2019).

Auxin	Cytokinin	Concentration	Explant	Response	Reference
2,4-D	Kinetin	1.0 + 1.0 mg/L	Shoot tip, leaf, stem, root	Produced profuse callus; highest callus biomass reported	Taha et al. (2008)
NAA	BAP	3.0 + 3.0 mg/L	Basal leaf with petiole	Gave the highest dry weight and optimum level of callus induction	Rahman et al. (2019)
NAA	Kinetin	1.0 + 0.1 mg/L	Anther	Approximately 86% callus induction	Bansal et al. (2023)

Auxin	Cytokinin	Concentration	Explant	Response	Reference
2,4-D	Kinetin	1.5 + 1.5 mg/L	Stem, leaf	100% callus induction; stem more responsive than leaf	Desouky et al. (2026)
2,4-D	BAP	1.5 + 1.5 mg/L	Leaf	Optimum level of callus induction	Mekky et al. (2018)
NAA	BAP	1 + 1 mg/L	Leaf	Successful callus induction	Verma et al. (2012)

Table 2. Plant growth regulator combinations reported for callus induction in *Catharanthus roseus*.

6 Factors Affecting Callus Growth and Morphogenesis

Beyond induction, how a callus culture grows and whether it differentiates further in *C. roseus* depends on explant source, PGR regime, environmental conditions and general culture management. Together, these variables set the pace of cell division, how much biomass accumulates, and whether the tissue has any real potential for organogenesis or somatic embryogenesis — which is why tuning culture conditions carefully is so important for keeping cultures healthy and actively growing (George et al., 2008; Phillips & Garda, 2019; Verma et al., 2012).

6.1 Explant Type and Physiological Age

Both the type of explant and its physiological age at excision have a marked effect on how well callus forms and grows afterward. Younger, juvenile tissue generally shows greater morphogenic competence than mature tissue, largely because of its more active metabolism and higher endogenous hormone levels. In *C. roseus* specifically, stem, leaf, shoot tip, hypocotyl, root and floral explants have all shown callogenic responses that vary with developmental stage and physiological condition (Taha et al., 2008; Wang et al., 2012; Desouky et al., 2026; Kim et al., 2004).

6.2 Environmental Conditions

Environmental factors exert a strong influence over how callus proliferates and differentiates.

Temperature. Most cultures of this species are kept at 24–27°C, a range that favours good cell division and steady biomass accumulation; temperatures well outside this window tend to suppress growth and can trigger tissue browning (George et al., 2008; Taha et al., 2008; Rahman et al., 2019; Desouky et al., 2026).

Light intensity and photoperiod. Callus is often initiated in the dark, then shifted to a 16-hour light / 8-hour dark cycle once growth and differentiation are underway. Light exposure at this stage affects chlorophyll development, metabolite production and morphogenic outcomes more broadly (Phillips & Garda, 2019; George et al., 2008; Akhar & Bagheri, 2013).

6.3 Culture Conditions

How the culture is managed day to day also shapes performance.

Incubation period. *C. roseus* callus is generally visible within 2–4 weeks of inoculation, with the exact timing depending on both the explant and the PGR combination used, and this window also affects the eventual size, texture and regenerative capacity of the tissue (Desouky et al., 2026; Rajora et al., 2013; Kalidass et al., 2010).

Subculture frequency. Transferring callus to fresh medium every 3–4 weeks keeps nutrient depletion and toxic metabolite build-up in check, which in turn preserves growth vigour over the longer term (Kalidass et al., 2010; George et al., 2008).

7 Characterization of Callus in Catharanthus roseus

Explant	PGR combination (mg/L)	Callus colour	Callus texture	Remarks	Reference
Leaf	NAA (3.0) + BAP (3.0)	Green	Compact	Yielded significant fresh and dry weight	Rahman et al. (2019)
Leaf	2,4-D (1.5) + Kinetin (1.5)	Creamy	Friable	Yielded high catharanthine and vinblastine concentrations	Desouky et al. (2026)
Stem	BAP (1.0) + NAA (1.0)	Green	Compact	Yielded high vincristine concentrations	Desouky et al. (2026)
Stem	2,4-D (0.2) + Kinetin (0.2)	Greenish yellow	Friable	Maximum amount of dry biomass	Kalidass et al. (2010)
Anther	NAA (1.0) + Kinetin (0.1)	White to pale yellow	Friable	Suitable for phytochemical studies	Bansal et al. (2023)
Immature zygotic embryo	2,4-D (1.0)	Off white	Friable	Embryogenic callus developed after subculture; suitable for suspension culture	Kim et al. (2004)
Root	NAA (1.5) + Kinetin (0.1)	Creamy	Soft	Yielded significant fresh and dry weight	Akhar & Bagheri (2013)

Table 3. Characterization of callus in *Catharanthus roseus* reported by different studies

Taken together, the published record shows that *C. roseus* callus varies quite a bit in colour, texture and growth habit depending on which explant and which PGR combination were used. Cream, white, pale yellow and green calli show up most often. Friable callus tends to be favoured for starting suspension cultures and for metabolite production generally, while compact callus more often shows enhanced organogenic and regenerative behaviour. Green colouration usually signals active chlorophyll development and healthy growth, whereas browning is typically read as a sign of senescence or phenolic oxidation. Stem-derived callus, in most reports,

out-produces leaf-derived callus in terms of raw biomass (Kalidass et al., 2010; Bansal et al., 2023; Desouky et al., 2026; Kim et al., 2004; Rahman et al., 2019; George et al., 2008).

8 Applications of Callus Culture in Catharanthus roseus

Callus culture in *C. roseus* has found its way into a fairly wide range of biotechnological and pharmaceutical uses. Its most obvious application is producing the terpenoid indole alkaloids vinblastine and vincristine, both valued for their anticancer activity (Mishra & Verma, 2017). Callus is also a convenient platform for elicitor-based work, where compounds such as methyl jasmonate and salicylic acid are added specifically to raise alkaloid accumulation (Razzaq et al., 2025; Paul et al., 2026; Siddiqui et al., 2013). Beyond metabolite production, callus cultures support plant regeneration and micropropagation, giving researchers a route to disease-free, genetically uniform plant material (Taha et al., 2008; Singh et al., 2011; Pietrosiuk et al., 2007), and they play a supporting role in genetic transformation and metabolic engineering work aimed at improving alkaloid biosynthesis pathways (Wang et al., 2012). Friable callus in particular can be used to establish cell suspension cultures for larger-scale biomass and metabolite production, and more recent work has begun exploring its use in the green synthesis of nanoparticles for biomedical applications (Osibe et al., 2018; Kim et al., 2004).

9 Challenges in Callus Culture of Catharanthus roseus

For all its usefulness, callus culture in *C. roseus* still runs into a fairly consistent set of problems. Chief among them is that in vitro cultures generally produce vinblastine and vincristine at lower and less consistent levels than field-grown plants do (Almagro et al., 2015). Extended subculturing brings its own risk of somaclonal variation, which can leave cultures genetically and biochemically unstable over time (Ikeuchi et al., 2013). Tissue browning and necrosis, driven by phenolic oxidation, is another recurring issue that can undercut both viability and growth. Microbial contamination — bacterial and fungal alike — remains a persistent obstacle at the culture-establishment stage, particularly when explants come straight from field-grown plants rather than a sterile stock. And because induction and morphogenesis depend so heavily on explant type, genotype and PGR combination, standardising protocols across different cultivars and laboratories has proved genuinely difficult (George et al., 2008; Desouky et al., 2026; Ikeuchi et al., 2013). Scaling any of this up to commercial production adds a further layer of difficulty: growth rates stay slow, metabolite yields stay modest, and sterile culture conditions are expensive to maintain at scale. Addressing these limitations will likely require continued work on elicitation strategies, metabolic engineering approaches, and bioreactor technology.

10 Future Perspectives

Future work on *C. roseus* callus culture will probably centre on raising TIA yields — vinblastine and vincristine especially — through more advanced biotechnological approaches. Elicitors, metabolic engineering, and genome-editing tools such as CRISPR/Cas systems all offer plausible routes to higher alkaloid biosynthesis and may help get around some of the limits of conventional culture systems (Das et al., 2025; Guo et al., 2025). Bringing in omics approaches — genomics, transcriptomics, proteomics and metabolomics together — should also help clarify the molecular mechanisms that govern callus growth and secondary metabolite production in the first place (Guo et al., 2025). Cell suspension cultures and bioreactor-based systems, meanwhile, could support genuinely large-scale production of bioactive compounds under tightly controlled

conditions (Verma et al., 2013; Alcantara et al., 2021). There is also room to explore explants that have received comparatively little attention so far, particularly floral tissues such as petals and ovaries, which may turn out to have their own explant-specific responses and metabolite profiles worth characterising (Desouky et al., 2026). Combining callus culture with nanotechnology and stress-based elicitation strategies is another avenue that could raise both metabolite yield and commercial viability (Tonk et al., 2016; Siddiqui et al., 2013; Desouky et al., 2026). If these lines of work pan out, they could position C. roseus callus culture as a genuinely sustainable biofactory for high-value pharmaceutical compounds.

11 Conclusion

Catharanthus roseus occupies an important place both as a medicinal plant in its own right and as a working model for plant tissue culture research, thanks to its capacity to produce pharmacologically significant TIAs including vinblastine and vincristine. Callus culture has become one of the more effective in vitro techniques for studying growth, regeneration and secondary metabolite production under controlled conditions in this species. How successfully callus is induced depends on a cluster of interacting factors — explant type, medium composition, PGR combination and environmental conditions among them. Across the explants that have been tested — leaves, stems, shoot tips, roots, hypocotyls and floral tissue — callogenic responses differ considerably depending on the specific conditions used. The literature converges reasonably well on MS medium with an appropriate auxin–cytokinin pairing, particularly 2,4-D with kinetin or NAA with BAP, as the standard approach for efficient induction and proliferation. Characterising the resulting callus by colour, texture, growth pattern and embryogenic potential gives useful information about how well a given culture is performing and what its regenerative prospects look like. Callus cultures have, over time, been put to use in secondary metabolite production, genetic transformation, plant regeneration, elicitation studies and several other biotechnological applications. Even so, problems remain: alkaloid yields stay low relative to field plants, somaclonal variation crops up with extended subculturing, browning and contamination continue to interfere with culture establishment, and scaling up to commercial volumes remains genuinely difficult. Future research will need to bring together elicitation strategies, metabolic engineering, omics approaches and bioreactor systems if metabolite production and culture efficiency are to improve substantially. On balance, callus culture still looks like one of the more promising routes toward conserving, improving and sustainably exploiting C. roseus, with real potential for further pharmaceutical and biotechnological application down the line.

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